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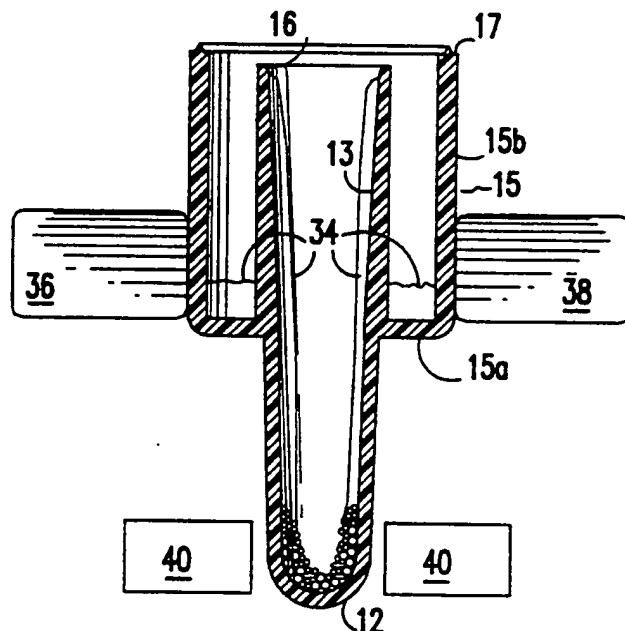
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(54) Title: IMPROVED CENTRIFUGE VESSEL FOR AUTOMATED SOLID-PHASE IMMUNOASSAY



A centrifuge vessel (10) for performing automated immunoassays comprises a center tube (11), an outer waste chamber (15), and a plurality of microparticle beads (32) housed within the center tube (11). The microparticle beads (32) have a magnetizable core which are acted upon the external magnetic sources (40) during washing operations.

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IMPROVED CENTRIFUGE VESSEL FOR AUTOMATED SOLID-PHASE IMMUNOASSAY

DESCRIPTION

5

BACKGROUND OF THE INVENTION

Field of the Invention

10 The present invention is generally related to
a centrifuge vessel which is rotated at high speed
about its longitudinal axis and, more particularly,
to a centrifuge vessel which can be used to
separate an analyte from a biological solution in
15 an automated fashion.

Description of the Prior Art

 Centrifuges are well known commercial and
laboratory tools that are used to separate
20 materials of varying densities. Centrifuges are
often used for clarifying liquids whereby suspended
solids such as cells or proteins are sedimented
from solution by high speed rotation of the
centrifuge vessel. Two common types of centrifuges
25 are the horizontal centrifuge and the fixed angle
centrifuge.

 In the conventional horizontal centrifuge,
sample containers of the solution to be centrifuged
are placed in holders called "buckets". These
30 buckets are subsequently attached to a vertical
rotor in a balanced arrangement and swung to a
horizontal plane under centrifugal force. While
being rotated at high speeds in the horizontal

5 position, the denser particles in the sample move along an unimpeded path toward the bottom of the buckets to form a smooth, even "pellet" of sedimented material. The non-pelleted supernatant solution can be decanted from the bucket once centrifugation is halted.

10 In the conventional fixed angle centrifuge, a rotor holds a plurality of sample containers at fixed angles relative to the axis of rotation. In the same fashion as in the horizontal centrifuge, at least a pair of sample containers must be used in order to balance the rotor during high speed spinning. During high speed rotation of the fixed angle rotor, the denser particles in the sample
15 containers pellet along the side wall of the container. Pelleting on the side wall makes decanting of the supernatant more difficult because of the possible resuspension of the pellet; however, there are certain advantages in using the
20 fixed angle centrifuge. For example, in the fixed angle centrifuge there is a shorter path for sedimentation resulting in a shorter time for separation and, more importantly, higher speeds of rotation are achievable with fixed angle rotors
25 because they are subjected to less air turbulence than the hanging bucket centrifuges. Higher rotation speeds allow greater centrifugal forces to act on the suspended solids.

30 As pointed out above, in both the horizontal and the fixed angle centrifuges, the rotor must be balanced accurately in order to prevent damage to the centrifuge. In order to properly balance the rotor, the lab technician must precisely weigh each

centrifuge sample container using a balance and then position two sample containers of equal weight in the rotor on opposite sides. This balancing step is very tedious and often requires withdrawing and adding back sample to the sample container until it reaches the desired weight.

In United States Patent No. 4,639,242 to Babson, the complete disclosure of which is incorporated herein by reference, a vessel was described which allowed for the complete physical separation of a precipitate and supernatant in a single tube. In operation, a precipitating agent is first mixed with the biological specimen and given time to react with analyte therein. Then the tube is rotated about its longitudinal axis at high speed. During high speed rotation, the contents of the tube is forced against the inner wall of the tube and moves upward towards the top of the tube due to the tube's inclined inner surface. At the top of the tube, the precipitate is deposited within V-shaped grooves on the tube's interior. After rotation is stopped, the precipitate is retained in the V-shaped grooves at the top of the tube while the liquid drops back to the bottom of the tube.

U.S. Patent No. 4,639,242 to Babson also discloses a procedure for using the tube whereby a radioactively labeled antibody is bound in the bottom of the tube. The radioactively labeled antibody reacts with an analyte of interest in the biological fluid during a short incubation time during which the biological fluid is permitted to mingle with the bound antibody so that it may bind

the analyte of interest. Then the tube is rotated at high speed, thereby causing the fluid in the tube to rise to the top while the analyte remains bound to the antibody in the bottom of the tube. The radioactivity in the bottom of the tube is counted while the tube is spinning.

While the tube disclosed in U.S. Patent No. 4,639,242 to Babson is more adaptable to an automated processing environment than conventional horizontal and fixed angle centrifuges because it does not require the batch step of balancing pairs of rotor tubes, the Babson tube is not suitable for some of today's processing environments. For example, in some environments it may not be desirable to have a precipitation step. Not only does precipitation require extra chemicals to be used and time for the precipitation reaction to take place, but in some environments precipitation may reduce yield or the precipitating agent itself may affect the measured results. In addition, it may be desirable to stop the tube from spinning and use other instrumentation to make radioactive, fluorescent, or other related readings.

SUMMARY OF THE INVENTION

It is therefore an object of the present invention to provide a centrifuge tube which includes a means for collecting biological fluid and wash liquid during rotation about its longitudinal axis and for storing that biological fluid and wash liquid separate from a bound analyte after the rotation of the centrifuge tube has

ceased.

5 It is another object of this invention to provide a centrifuge tube which includes a waste chamber about its periphery, a cap over its top portion which allows fluid communication between the centrifuge tube and the waste chamber, and a biological material positioned within the centrifuge tube and being restricted from the waste chamber which is capable of binding an analyte of interest present in a biological sample.

10 It is yet another object of this invention to provide a centrifuge tube with a waste chamber secured about its periphery in fluid communication therewith which utilizes magnetizable microbeads having a biomaterial capable of binding an analyte bound thereto in the centrifuge tube.

15 According to the invention, a centrifuge tube has been developed which is suitable for performing automated solid phase immunoassays. As a general rule, immunoassays in the clinical laboratory have rapidly replaced other methods used to detect or quantitate substances in body fluids with important biologic or pharmacologic properties. The high levels of sensitivity and specificity achieved with immunoassays result from the specific, high-affinity reversible binding of antigens to antibodies, and from the development of methods for attaching readily detected labels (radioactive isotopes, fluorescent or chemiluminescent molecules, enzymes and the like) to antigens or antibodies. Although radioactive isotopes have been the most extensively used label, they are not preferred because of concerns with radioactivity.

Because of these concerns, the number of sensitive, specific immunoassays employing non-radioactive labels is rapidly expanding.

5 As is generally known among clinicians, many immunoassay procedures are based upon the reaction of an antigen, which is the analyte to be ultimately measured, with an antibody which has been adsorbed or otherwise bound to a solid surface. These solid surfaces (hence the
10 designation "solid-phase immunoassays") may be the interior of small test tubes (such as those available from Micromedex Systems, Inc. of Horsham, Pennsylvania), microtiter trays (such as the 96 well trays available from Amersham International of
15 Bucks, England), macrobeads (such as those available from Abbott Laboratories of Abbott Park, Illinois), or microparticles (such as those available from Pandex Laboratories, Inc. of Mundolein, Illinois), or magnetic particles (such
20 as those available from Corning Medical of Medfield, Massachusetts). An advantage of solid-phase immunoassay over liquid-phase immunoassay is that common reagents and serum constituents which can potentially interfere with the measurement of
25 the label are removed in the washing step of the procedure. While a variety of immunoassay schemes involving a solid-phase reactant are possible, most schemes utilize a solid-phase antibody and are generally classified as competitive or
30 noncompetitive.

In competitive immunoassays, which are generally used for small molecular weight analytes with only a single antibody binding site, antigen

present in the sample or standard competes with a measured amount of labeled antigen for a limited number of binding sites on a solid-phase bound antibody. After removal of any unreacted antigen from the test system, and washing the solid support material, the bound label is quantitated by suitable means well known in the art. The amount of labeled antigen bound to the solid-phase antibody is inversely related to the concentration of antigen (analyte) in the sample or standard.

In noncompetitive immunoassays, an excess of labeled antibody binds to essentially all of the analyte present in the sample, and an excess of the solid-phase antibody also binds to additional sites on the analyte. These two separate antigen-antibody reactions can be conducted either sequentially, by providing an intermediate washing step if the first reaction is with solid-phase antibody, or simultaneously. After the immunological reactions are allowed to reach equilibrium, the excess labeled antibody is removed, the solid phase is washed and the bound label quantitated by suitable means well known in the art. This type of immunoassay is also called a "sandwich assay" because the antigen is sandwiched between the solid-phase and labeled antibodies. It is also called an "immunometric assay" because the amount of label bound is usually a direct and linear function of the antigen concentration within the sample. It is also called a "two-site immunoassay" if the labeled and solid-phase antibodies are directed to distinct antigenic determinants on the analyte. This type of assay

can only be used with large molecular weight analytes with multiple antibody binding sites.

In an alternative sandwich assay format, the second antibody is unlabeled and the procedure is expanded to include an incubation of the sample with an excess of labeled third antibody specific for the IgG of the animal species from which the second antibody is elicited. In this instance, the immobilized and second antibodies are obtained from different animal species, in order to prevent the binding of labeled third antibody directly to the immobilized antibody. An advantage of this approach is that a single labeled antibody can serve as a common reagent for a number of analytes.

Common to all solid-phase immunoassays is the requirement that all unbound labeled antigen or antibody must be removed by thorough washing of the solid-phase prior to measurement of the label. Washing is a cumbersome procedure, particularly for automated analytical systems. For example, washing coated tubes or microwells by alternately adding and aspirating water or wash solution, as is customarily done, is inefficient since there is always some residual solution in the tube or well after each wash cycle. As many as four to six washes of about 4 ml are required to adequately wash the coated tubes conventionally used in present day immunoassay procedures. Furthermore, provisions must be made to collect all the used wash solutions because they could possibly contain infectious agents from the sample specimens.

The centrifuge vessel according to the present invention offers a number of advantages over the

present protocols and apparatus conventionally used in solid-phase immunoassay procedures. The centrifuge vessel includes a central tube member and a peripheral waste chamber. In operation, the analyte being tested for reacts with and is bound to a solid support positioned in the central tube and the excess sample fluid, reagents, and wash fluid are separated from the bound analyte by rotating the tube rapidly about its longitudinal axis. High speed rotation causes the fluids to move up the inclined wall of the central tube and droop into the peripheral waste chamber. Rotation continues until no fluid residue remains in the bottom of the tube. After rotation of the tube is halted, the excess sample fluid, reagents and wash fluid are safely stored in the waste chamber and do not re-mix with the bound analyte in the central tube.

A particular advantage of the present design is that multiple washings of the bound analyte can be performed in a sequential fashion as rapidly as the wash solution can be pipetted into the central tube of the vessel. The ability to wash the bound analyte in the rapid fashion results from the excess fluids being transferred to the waste chamber almost instantaneously because of the high speed centrifugal forces acting on the centrifuge vessel. Because of the speed with which washing is accomplished, multiple vessels may be processed sequentially without impairing sample throughput. The speed of processing the vessels allows for the precise control of the incubation times required by immunoassays, and allows for the normally labor-

intensive immunoassay procedure to be automated.

In a particular embodiment of the invention, the solid support is comprised of microparticles having a magnetizable core and a polymeric overcoating to which the biomaterial for binding the analyte in the biologic fluid is bound. The microparticles have the advantage of providing a cummulatively large surface area for binding interactions and faster kinetics. In operation, the biologic fluid is deposited in the centrifuge vessel and the microparticles present in the centrifuge tube become dispersed in the biologic fluid. After a sufficient reaction period, the biologic fluid is removed from the centrifuge tube by high speed rotation about its longitudinal axis and the microparticles are washed by pipetting water into the centrifuge tube and removing the water by high speed rotation. During high speed rotation of the centrifuge tube about its axis, the microparticles are held within the centrifuge tube by an external magnetic force exerted on the magnetizable cores of the microparticles. The present invention greatly improves the utility of microparticles in automated immunoassay environments because it provides a device which will allow rapid and thorough washing. In the past, microparticles have been very difficult to wash because of trapped interstitial fluids between adjacent microparticles. The high "G" forces imparted to the microparticle beads in the vertical direction which are developed during rotation about the central axis helps remove the trapped interstitial fluids.

BRIEF DESCRIPTION OF THE DRAWINGS

The foregoing and other objects, aspects and advantages will be better understood from the following detailed description of a preferred embodiment of the invention with reference to the drawings, in which:

Figure 1 is an isometric view of a centrifuge vessel;

Figure 2 is a cross-sectional side view of the centrifuge vessel of Figure 1;

Figure 3 is a cross-sectional side view of a cap which fits on the centrifuge vessel;

Figure 4 is a top view of the cap of Figure 3;

Figure 5 is a cross-sectional side view of a centrifuge vessel according to the present invention wherein microparticle beads are used as the solid support; and

Figure 6 is a cross-sectional side view of the centrifuge vessel of Figure 5 being rotated about its longitudinal axis at high speed where the microparticle beads are held in place by a magnetic force as fluid is expelled.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE INVENTION

Referring now to the drawings, and more particularly to Figures 1 and 2, which show the centrifuge vessel 10 comprises a longitudinally extending central tube 11 opened at the top 16 of its upper end portion 13, and sealed at its lower end portion 12. Integrally attached to tube 11 at

5 a point between the upper and lower ends is a generally cylindrical waste chamber 15 having a circumferentially extending bottom portion 15a extending between tube 11 and the waste chamber's upwardly extending cylindrical side wall 15b. The waste chamber 15 is open at its uppermost end 17 at a point above the top 16 of the tube 11. The waste chamber is coaxial with the tube 11, and has an inner diameter greater than the outer diameter of the tube 11.

10 Although the centrifuge vessel 10 may be manufactured from various materials, for ease of manufacture it is preferred that the vessel be molded from a plastic (polymeric) material which is compatible with its intended use. The uppermost end 17 of waste chamber 15 may be sealed with a metal foil or polymeric membrane 19 to contain the liquid in the vessel as it is spun on its longitudinal axis or, it may, as will be discussed in conjunction with Figures 3-4, have a cap welded thereto. If a foil membrane 19 is used, the center of the membrane 19 is punctured to allow addition of the sample and reagents to tube 11. If a welded cap is used, a small foil cover over the central tube 11 is removed to allow the addition of the sample and the reagents.

25 Figures 3 and 4 show a hard cover cap 20 which may be used instead of the polymer or foil film 19 to cover the open end 17 of waste chamber 15. The cap 20 has an outer flange 22 which fits inside and adjacent the inner diameter of sidewall 15b of waste chamber 15 and an outer welding region 24 which is welded to the top 17 of waste chamber 15.

Welding can be accomplished using traditional heat staking or ultra-sonic technologies and the preferred welded joint is called a shear seal. It is anticipated that other suitable techniques, such as gluing, could be used for applying the cap 20 to the top 17 of waste chamber 15. The cap 20 may include an inner flange 28, about the periphery of opening 30, that projects down into the top portion 13 of the center tube 11. The diameter of the inner flange 28 is smaller than the diameter of the top portion 13 of the center tube 11 so that fluid can pass between the flange 28 and the center tube 11, then up over the top 16 of the center tube and into the waste chamber 15. The advantages of welding a cap 20 to the centrifuge vessel 10 instead of applying a film 19 over the waste chamber 15 include allowing the centrifuge vessels 10 to be individually pressure tested for leaks prior to use and avoiding the additional processing step of trimming each foil or polymer film 19 to fit neatly on the centrifuge vessel 10. Pre-pressure testing the centrifuge vessels 10 is believed to be important since the vessels 10 are subjected to intense pressure during the high speed spin.

With reference back to Figures 1 and 2, the lower end 12 of the vessel 10 may be formed with a drive engagement means 14 that would allow a rotating axle member (not shown) to engage and spin the vessel 10 about its longitudinal axis. As depicted in Figure 2 (although the depiction is for illustration purposes only and the actual engagement means may take other forms), a female

5 socket 14 is adapted to fit in frictional
engagement about a male rotor axle (not shown) to
provide a union with the spinning rotor axle and
thereby provide for the spinning of the vessel
about its longitudinal axis. It is also possible
to spin the centrifuge vessel 10 about its
longitudinal axis using a set of three opposing
rollers (see rollers 36 and 38 in Figure 6) which
will engage the side wall of central tube 11 or the
10 side wall 15b of waste chamber 15. If the three
roller arrangement were used, the centrifuge vessel
10 would simply be placed between the rollers and
at least one of the rollers would be driven at high
speed.

15 As the vessel is spun about its axis, any
fluid within the central tube 11 will travel upward
along the inner surface of the tube 11. The inner
surface of the tube 11 is preferably manufactured
in such a manner so that the closed bottom 12 of
20 the tube 11 has a slightly smaller inner diameter
than the open top 16. The outward flaring of the
inner surface of the tube 11 provides an incline
which aids in transporting fluid upward which is
driven thereagainst under the centrifugal force.
25 As can be seen in the Figure 2, the uppermost
terminal end 16 of tube 11 is short of the cross-
sectional plane passing through and defining the
open uppermost end 17 of the chamber 15. This
height differential provides a passage which allows
30 fluid to pass from tube 11 into waste chamber 15.
Waste chamber 15 may also be manufactured with its
inner diameter at the bottom portion 15a being
slightly greater than its inner diameter at its

uppermost end 17, thereby functioning to hold fluid at a position towards the base of waste chamber 15 as the centrifuge vessel 10 is being spun about its longitudinal axis. The centrifuge vessel 10 does not need to be removed from the drive means to discard biological or wash fluid between washings since all of the wash fluid is transported to and held in waste chamber 15. Therefore, multiple washings are performed simply by repetitively pipetting wash fluid into the vessel 10 and discarding the waste wash fluid by rotating the centrifuge vessel 10 about its longitudinal axis at high speed.

When the centrifuge vessel 10 is being used in immunoassay procedures, the bottom inner portion of the tube 11 may be coated with an antigen or antibody protein (not shown) which binds to the inner surface of the tube 11. This coating preferably extends from the very bottom of the closed end 12 of the tube 11 upwards to a point anywhere less than about 25% of the overall length of tube 11 where the actual length of the coating is a matter of design of the coating process for manufacture of the coated tube. As an alternative to coating the inner surface of the tube 11 with a specific antigen or antibody, or as a means for providing for additional reaction surfaces for the desired immunoassay procedure, a solid support 18 can be placed within the tube 11 where the solid support includes a binding surface, such as a bound antigen or antibody molecule, for selectively binding a specific analyte in the biological fluid under test. Suitable solid supports 18 may include

antigen or antibody coated spheres of organic polymers or inorganic polymers. Silica gels are a typical example of inorganic polymers used as binding spheres. Suitable solid supports 18 are commercially available or can be easily manufactured and the choice of support 18 is dependent upon the binding characteristics of the appropriate antigen or antibody or the ligand used to bind the antigen or antibody to the support 18.

The present patent application is directed to an improvement, wherein microparticle beads 32 (see Figures 5 and 6) having a magnetizable core, a polymeric overcoat, and biomaterial bound to the polymeric overcoat for binding analyte in the biologic fluid are used in the centrifuge vessel. The microparticle beads 32 provide surface area and faster kinetics advantages in conducting immunoassays.

When conducting immunoassays, a sample of fluid containing the analyte to be determined is added to the vessel's central tube 11, along with a solvent if required. The microparticle beads 32 can be added to the vessel's central tube 11 as a suspension. If a competitive immunoassay is being performed, the labeled antigen is also added to the central tube 11. Addition of the sample, solvent, microparticle beads 32 and any other reagents can be achieved using a pipette probe or a hypodermic needle. It is contemplated that the additions can be made in an automated fashion using a machine which performs the pipetting. The tube 11 contents are then mixed by lateral agitation or by slowly rotating the vessel 10 about its

longitudinal axis. During mixing, the analyte in the sample fluid binds to the antibody on the microparticle beads 32 at the base 12 of the center tube 11. After an appropriate incubation period, the vessel 10 is rotated about its longitudinal axis at a speed sufficiently great to cause any fluid within the central tube 11 to be transferred into the waste chamber 15. Water or other wash fluids may be intermittently introduced into the central tube 11 to wash any remaining sample and reagent out of the tube 11 and into the waste chamber 15. Washing can be performed by stopping the tube 11, adding the water, and then starting the rotation of the tube 11 again, or by adding the water while the tube 11 is spinning. Once washing is completed, the bound label in the lower portion of the central tube 11 may then be quantitated by suitable means well known in the diagnostic arts.

Figures 5 and 6 illustrate a particular embodiment of the invention wherein microparticle beads 32 are used as the solid support in the base 12 of center tube 11. Microparticle beads 32 are available from a wide variety of sources including Seradyne, Bangs Laboratories, and Corning Medical Laboratories. The microparticle beads 32 have a magnetizable core, such as a ferrous material, and a polymeric overcoat, such as polystyrene or the like. The biomaterial used to bind the analyte in the biological fluid is bound to this polymeric overcoat in the same manner as it would be to the bottom portion 12 of tube 11 or to the macrobead 18 shown in Figure 2.

Like numbers in Figures 2, 5 and 6 indicate like elements. The principal difference between the microparticle beads 32 of Figure 5 and the macrobead 18 of Figure 2 is size. Specifically, microparticle beads 32 have a diameter ranging from microns to a few millimeters, whereas macrobeads are typically on the order of a quarter of an inch in diameter. The microparticle beads 32 have the advantage of providing a cummulatively large surface area for binding interactions and faster kinetics when conducting immunoassays. Figure 6 shows that in operation, the biologic fluid 34, after a being intermingled for a sufficient reaction period with the microparticle beads 32 having the bound biomaterial for binding analyte in the fluid 34, is removed from the center tube 11 by high speed rotation about its longitudinal axis. High speed rotation can be achieved using a drive roller 36 and free rollers 38 which contact the outside wall of the waste chamber 15 or by other means. The microparticle beads 32 stay within the center tube 11 via an external magnetic force exerted on the magnetizable cores of the microparticle beads 32. It is contemplated that the external magnetic force could be supplied by fixed magnets or electromagnets 40 located at a spin station which exert a force through the bottom portion 12 of center tube 11. The microparticle beads 32 are washed by pipetting water into the center tube 11 and removing the water by high speed rotation.

Microparticle beads 32 are viewed as an important solid support medium since they provide

enhanced reaction area per unit volume and faster reaction kinetics in conducting automated immunoassays. In the past, washing the bound biomaterial has been the most difficult step to automate. It is very difficult to thoroughly wash microparticle beads because biological fluid, which may contain free label, tends to become trapped in interstitial spaces. The present invention greatly improves the utility of microparticle beads 32 in automated immunoassay environments because it provides a device which will allow rapid and thorough washing. During high speed rotation of the center tube 11, high "G" forces in the vertical direction are developed which cause fluid in the interstitial spaces of the microparticle beads 32 to be evacuated. Washing can be repeated rapidly simply by pipetting more water in the center tube 11 followed by high speed rotation.

Of course, the vessel 10 according to the present invention may be used in other procedures besides immunoassays. For example, among the many techniques used today in biochemical separations, perhaps the most efficient and selective is affinity chromatography. Affinity chromatography does not rely on general molecular properties such as size, electrical charge or density to carry out a separation. Instead, it involves a very specific interaction between two biomolecules, one of which is chemically attached (in solid phase separations) to a solid support phase, and the other of which is dissolved in solution. Such interactions are almost a universal feature of biomolecules. Specific examples would include binding between

antibodies and antigens, lectins and antigens,
hormones and receptors, enzymes and either
substrates, coenzymes, inhibitors or activators,
DNA and its complement (a repressor or catabolite
5 gene activator for double-stranded DNA or the
complement of a single strand of DNA) and messenger
RNA and ribosomes. It will be readily apparent to
one skilled in the separation art, that the vessel
of the present invention may be modified by
10 chemically attaching an appropriate biomolecule to
the microparticle beads 32, and utilizing the
vessel 10 as an affinity chromatographic separation
device. Once the interaction between the two
biomaterials is completed, the vessel 11 may simply
15 be spun rapidly about its longitudinal axis to
remove the unbound biomaterial from the center of
the tube 11 into the waste chamber 15.

While the invention has been described in
terms of its preferred embodiment, which includes a
20 center tube having a peripheral waste chamber and a
bound antibody positioned in the center tube, those
skilled in the art will recognize that the
invention may be practiced with modification within
the spirit and scope of the appended claims.

25

CLAIMS

Having thus described our invention, what we
claim as new and desire to secure by Letters Patent
is as follows:

1. A centrifuge vessel designed for rotation at
high speed about a central vertical axis for use in
performing assays on liquid biological samples
introduced into said centrifuge vessel, comprising:

a tube having a conical interior wall tapering
outwardly from said central vertical axis as said
wall extends from a closed bottom end of said tube
to an open top end of said tube, wherein

centrifugal force generated as said centrifuge
vessel rotates at high speed about said central
vertical axis urges said liquid biological sample
to flow along said tapered wall toward said open
top end and causes said liquid biological sample to
spray from said open top end outwardly away from
said central vertical axis;

a plurality of microparticle beads, each
having a magnetizable portion and a biomaterial
attached thereto, for binding an analyte in said
biological sample positioned inside said tube but
not attached to said tube; and

means, secured to said tube, for catching and
holding said liquid biological sample sprayed from
said open top end of said tube when said centrifuge
vessel is at rest with respect to rotation about
said central vertical axis, said means for catching
and holding said liquid biological sample
preventing said liquid biological sample sprayed

from said open top end of said tube from flowing back into said tube when said centrifuge vessel is at rest.

5 2. A centrifuge vessel as recited in claim 1 wherein said means for catching and holding said biological sample sprayed from said top end of said tube comprises a waste chamber connected to said tube having a side wall coaxial with said tube and
10 a closed bottom end.

 3. An instrument for performing automated immunoassays, comprising:
 a centrifuge vessel designed for rotation at
15 high speed about a central vertical axis for use in performing assays on liquid biological samples introduced into said centrifuge vessel, said centrifuge vessel comprising

 (i) a tube having a conical interior wall
20 tapering outwardly from said central vertical axis as said wall extends from a closed bottom end of said tube to an open top end of said tube, wherein centrifugal force generated as said centrifuge vessel rotates at high speed
25 about said central vertical axis urges said liquid biological sample to flow along said tapered wall toward said open top end and causes said liquid biological sample to spray from said open top end outwardly away from
30 said central vertical axis,

 (ii) a plurality of microparticle beads, each having a magnetizable portion and a biomaterial attached thereto, for binding an

analyte in said biological sample positioned inside said tube but not attached to said tube, and

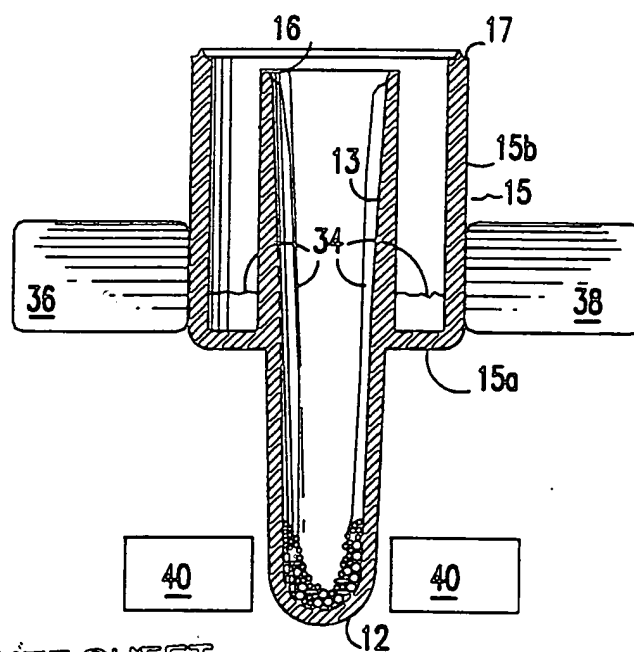
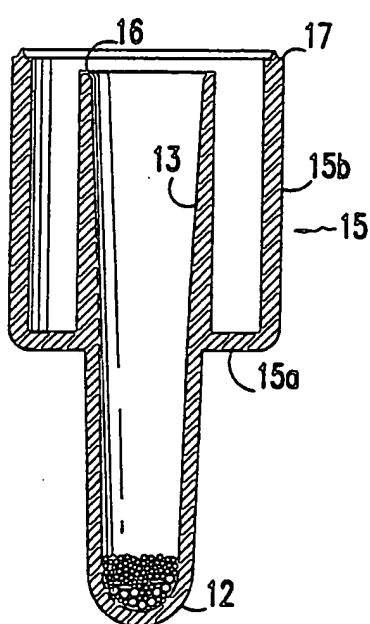
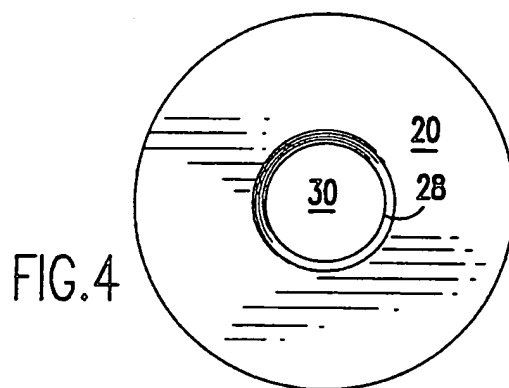
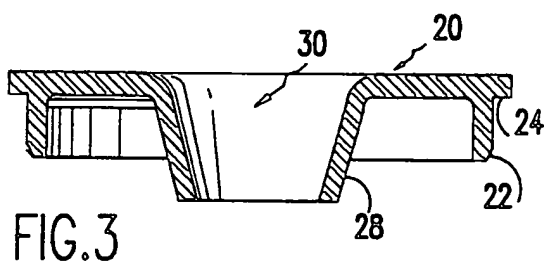
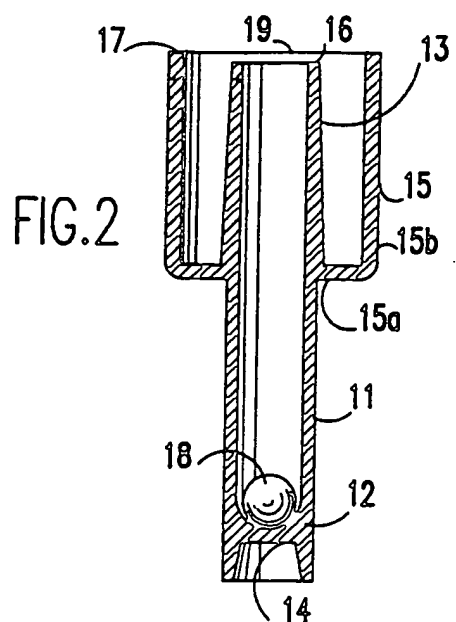
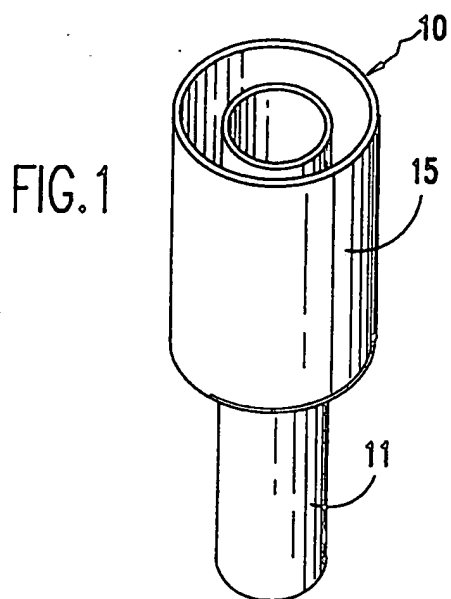
(iii) means, secured to said tube, for catching and holding said liquid biological sample sprayed from said open top end of said tube when said centrifuge vessel is at rest with respect to rotation about said central vertical axis, said means for catching and holding said liquid biological sample preventing said liquid biological sample sprayed from said open top end of said tube from flowing back into said tube when said centrifuge vessel is at rest;

means for rotating said centrifuge vessel about said central vertical axis; and

magnetic means for exerting a magnetic force on said plurality of microparticle beads in said tube of said centrifuge vessel during rotation of said centrifuge vessel.

4. An instrument as recited in claim 3 wherein said magnetic means is a permanent magnet.

5. An instrument as recited in claim 3 wherein said magnetic means is an electromagnet.



SUBSTITUTE SHEET

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10247**A. CLASSIFICATION OF SUBJECT MATTER**

IPC(5) : G01N 33/545; B04B 5/10, 1/06; B01L 3/14

US CL : 422/69, 72, 102; 436/526; 494/43, 44

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 422/69, 72, 102; 436/526; 494/43, 44

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y,P,&	US, A, 5,084,240 (Babson) 28 January 1992, see entire document.	1-5
Y	US, A, 3,985,649 (Eddelman) 12 October 1976, see entire document.	1-5
Y	US, A, 4,793,973 (Ringrose) 27 December 1988, see entire document.	1-5
Y	EP, A, 0,358,948 (LEE ET AL) 21 March 1990, see entire document.	1-5
A	US, A, 3,722,790 (Natelson) 27 March 1973.	1-5
A	US, A, 3,826,622 (Natelson) 30 July 1974.	1-5
A	US, A, 3,838,809 (Williams) 01 October 1974.	1-5
A	US, A, 4,115,534 (Ithakissios) 19 September 1978.	1-5
A	US, A, 4,314,968 (Guigan) 09 February 1982.	1-5
A	US, A, 4,458,812 (Dreier et al.) 10 July 1984.	1-5

☒ Further documents are listed in the continuation of Box C.
 ☐ See patent family annex.

°	Special categories of cited documents:	T	later document published after the international filing date or priority date and not in conflict with the application but cited to underpin the principle or theory underlying the invention
A	document defining the general state of the art which is not considered to be part of particular relevance	X	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
E	earlier document published on or after the international filing date	Y	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
L	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (to specified)	*A*	document member of the same patent family
O	document referring to an oral disclosure, use, exhibition or other means		
P	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search

10 February 1993

Date of mailing of the international search report

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US92/10247

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category ^a	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US, A, 4,470,954 (Chiknas) 11 September 1984.	1-5
A	US, A, 4,639,242 (Babson) 27 January 1987.	1-5
A	US, A, 4,933,291 (Dains et al.) 12 June 1990.	1-5
A	JP, A, 60-207058 (SHIMADZU SEISAKUSHO KK) 18 October 1985.	1-5